BBA 76058

STUDIES ON THE EFFECTS OF UNCONJUGATED DIHYDROXY BILE SALTS ON RAT SMALL INTESTINAL FUNCTION *IN VIVO*

G. E. SLADEN AND J. T. HARRIES*

Department of Gastroenterology, St. Bartholomew's Hospital, London, E.C.1 (Great Britain)

(Received May 24th, 1972)

SUMMARY

1. The inhibitory effects of the unconjugated dihydroxy bile salts deoxycholate and chenodeoxycholate on small intestinal transport of fluid, ions and glucose have been studied in the rat *in vivo*, using both closed loop and perfusion techniques. The results obtained with the two methods are in broad agreement, although some quantitative differences were observed.

2. In the jejunum transport was inhibited by 1 mM deoxycholate, and fluid and NaCl absorption abolished by both salts at 5 mM concentration. Glucose absorption was inhibited to a lesser extent. By contrast, the ileum was relatively insensitive to these bile salts, although slight but significant inhibition was produced by both at 5 mM concentration. Increased ileal secretion of K^+ in response to bile salts could not entirely be explained in terms of passive movement.

3. Histological damage to mucosal cells at villous tips was seen in jejunum and ileum in response to 5 mM (but not 1 mM) deoxycholate, but this was not associated with altered mucosal permeability.

4. Evidence from $^{22}Na^+$ fluxes and the maintenance of normal polarisation of the mucosa suggest that bile salts may promote a non-electrogenic movement of NaCl and fluid into the lumen, without primarily affecting the normal Na^+ absorption mechanism.

INTRODUCTION

In a previous report we have described the effects of various bile salts on small intestine transport of glucose, fluid and electrolytes in the rat *in vivo*, using a closed-loop technique[1]. The dihydroxy unconjugated salts, deoxycholate and chenodeoxycholate, inhibited transport in luminal concentrations of 1–5 mM, the jejunum being far more sensitive than the ileum. On the other hand, the trihydroxy salt cholate and the taurine conjugates of cholate and deoxycholate produced little or no inhibition. In this paper possible mechanisms of these inhibitory effects have been studied in more detail. For several practical reasons it was found that a continuous perfusion technique was more satisfactory than the closed loop method for these further

Abbreviations: PD, potential difference.

* Present address: Department of Child Health, Institute of Child Health, Guilford Street, London, W.C.1., England.

studies, and it was first necessary to demonstrate that the earlier findings could be confirmed with this method. The present results are in broad agreement, although some quantitative differences emerged. Using both techniques further information about alteration of electrolyte transport has been obtained.

METHODS

Male, Wistar rats, weighing 250–350 g, were starved for 12 h prior to the experiments, but allowed tap water *ad libitum*. Anaesthesia was induced with diethyl ether and maintained, in the prolonged perfusion experiments only, with intramuscular Nembuta (6–12 mg intramuscularly). The rectal temperature was maintained throughout the experiments at 37 °C by overhead electric lamps. The abdomen was opened with a midline incision and 20–30 cm lengths of proximal jejunum (from the duodenojejunal junction) or distal ileum (from the ileocaecal valve) were prapared. Faecal material was removed from the loops by washing with isotonic saline at 37 °C.

The closed loop studies have been described briefly elsewhere[1]. The washed loops were emptied by injection of air and careful manual pressure. A known quantity (2.5–3.0 ml) of test solution at 37 °C, measured by weighing, was instilled into the loop *via* an inlet catheter (Portex PP60) and the contents mixed manually. The loops were returned to the abdomen for an absorption period of 20 min (jejunum) or 30 min (ileum). These periods were found empirically to give consistent and easily measurable absorption in control studies. After the absorption period, the animal was killed and the loop dissected out and carefully blotted. The full loop was weighed, emptied and slit longitudinally to expose the mucosal surface, which was gently blotted. The empty loop was weighed before and after the mesentery was stripped from it, so that absorption rates could be expressed in relation to the wet weight of gut tissue. In these studies a Mettler P162 balance weighing to the nearest 1 mg was used. In our earlier paper[1] we have indicated that the weighing technique was preferred to the use of a theoretically non-absorbed marker such as polyethyleneglycol, because it was found that marker recoveries from closed loops were incomplete.

Two particular sources of error were assessed initially. First, it is obviously impossible to get the loop completely empty at the beginning of the experiment, so that the initial loop volume is greater than the instilled volume by an unknown amount. This will have the effect of underestimating absorption rates and overestimating secretion rates. To test the practical importance of this, a series of experiments were performed in which the rats were killed and the loops dissected out immediately after instillation of test fluid. Final loop volumes were expressed as a percentage of the instilled volume. In 6 jejunal loops the mean recovery was 102.7% (range 98.5–106.9%), and in 6 ileal loops 107% (range 104–112%). These figures mean that, on average, absorption rates are underestimated (and secretion rates overestimated) by 0.08 ml fluid and 11.2 μequiv Na^+ per g wet wt of jejunum and 0.2 ml fluid and 28 μequiv Na^+ per g wet wt of ileum, assuming a mean luminal Na^+ concentration of 140 mequiv/l. Absolute absorption rates at or near zero have therefore to be interpreted with caution. However, this error should not be affected by the nature of the instilled solution so that comparative data are quite valid.

The second source of error assessed was the use of 1 g wet wt as the reference unit in these studies (and in the perfusion studies to be described later). It is possible

that changes in the hydration of the mucosa or gut wall at different absorption rates of fluid could seriously affect the results. In a limited series of experiments the loops were also dried in an oven at 120 °C for 24 h, cooled in a dessicator and re-weighed. The wet and dry weights were then correlated in both control experiments and in those involving deoxycholate and chenodeoxycholate. Excellent correlation was obtained—in the jejunum $r = 0.922$ ($n = 33$ pairs) and in the ileum $r = 0.978$ ($n = 28$ pairs)—and the quantitative relationships were not affected by the nature of the luminal solution. These findings validate the use of wet weight as reference in both types of study.

In the perfusion studies the test fluid was infused continuously at 0.2 ml/min (Palmer injection apparatus) through a fine inlet catheter (Portex PP 60) inserted into the proximal end of the loop. A wider bore (2 mm) outlet tube (Portex 4/80) was inserted into the distal end for the continuous collection of effluent and the loop was returned to the abdominal cavity. This infusion rate was found empirically to give a satisfactory steady flow of effluent and readily measurable absorption rates. Flow from the effluent tube usually began after a delay of 10–20 min and an equilibration period of 60 min was allowed, followed by three consecutive 20-min collections. The rats were killed at the end of the 2-h perfusion and the loops dissected, stripped of mesentery and weighed. In these studies polyethyleneglycol was used as an unabsorbable marker and two major assumptions are involved – that a steady state is set up, and that the marker is indeed not absorbed.

In a series of 34 perfusions, the variation of triplicate polyethyleneglycol concentrations in the effluent (analytical method—*vide infra*) was expressed by the range as a percentage of the mean. These individual variations were lognormally distributed with a range of 1.1–18.1% (mean 5.4%). The variation was greater than 10% in only 6 of the 34 studies, indicating that an acceptable steady state was achieved in the majority of the studies. In 36 perfusions, the 20-min effluent volumes were weighed so that the total polyethyleneglycol output during the second hour could be accurately measured. This was then compared with the known hourly input of polyethyleneglycol—the pump rate being checked at the beginning and end of each experiment. In 13 ileal perfusions, the output of polyethyleneglycol expressed as a percentage of input ranged from 93.8–117% (mean 107%). In 23 jejunal perfusions the range was 85.4–113% (mean 106.6%). These findings indicate that polyethyleneglycol is more than adequately recovered in this steady-state system although the reason for the mean recoveries being somewhat greater than 100% is not clear. There was no difference in recovery pattern between the various solutions perfused, although in the jejunum mean polyethyleneglycol recovery from the control solutions was 105% (range 94.6–110.5%, $n = 7$) compared with a mean of 97.2% (range 85.4–108.5%, $n = 8$) for the perfusions containing 5 mM deoxycholate. This difference was not statistically significant ($P = 0.3$), so that there is no evidence in these studies that the bile salt interferes with polyethyleneglycol recovery. It was considered important to exclude this possibility in view of the report that higher concentrations of deoxycholate promote the absorption of phenol red (often used as another "non-absorbable" marker) by the rat intestine *in vivo*[2].

In the majority of the perfusion studies, the mucosal potential difference (PD) was measured by a DC multimeter Type TM 9 B (Levell Electronics, High Barnet, Herts). The luminal electrodes (silver/silver chloride electrodes, built into 5-ml

disposable syringes filled with isotonic saline) were inserted *via* a No. 12 needle into the inlet tubing near the point of entry into the gut. A similar serosal electrode was suspended in the peritoneal cavity, which was kept filled with normal saline. In general, PDs changed little during the second hour of the perfusion—measurements were noted every 5–10 min, and a single mean value was obtained for each experiment.

In some studies the loops were washed with saline at the end of the experiment and a small sample of tissue from the middle of the segment was immersed in formalin (10% in normal saline) for subsequent light microscopical examination. A correction for weight lost was made from length–weight correlations obtained from many previous studies.

Cardiac puncture was performed at the end of many studies and plasma separated for subsequent analysis of electrolytes, glucose and, where relevant, isotopic counting.

Composition of solutions used

The solutions were isotonic (approx. 285 mosmoles/kg), were buffered to pH 7.4 by phosphate (2 mM NaH_2PO_4, 14 mM Na_2HPO_4) and the perfusion solutions contained polyethyleneglycol (3 g/l). In the jejunum, glucose containing (20 mM) and glucose free solutions were used, whereas none of the ileal solutions contained glucose. All solutions contained KCl (4 mM) and the bile-salt solutions contained varying concentrations of sodium deoxycholate or sodium chenodeoxycholate up to 5 mM. These bile salts were obtained from Maybridge Research Chemicals, Launceston, Cornwall, and were checked for purity by thin-layer chromatography. Residual tonicity was made up with NaCl: the final Na^+ concentration was 140 mequiv/l in the presence, and 150 mequiv/l in the absence of glucose, the Cl^- concentration in control solutions being 114 and 124 mequiv/l, respectively.

In the closed loop studies $^{22}Na^+$ (Radiochemical Centre, Amersham, Bucks) was added in a concentration of 2.5 μCi/l. To the solutions containing 5 mM chenodeoxycholate, 2.5 μCi/l of [*carboxy*-^{14}C]chenodeoxycholate (International Chemical and Nuclear Corporation Tracerlab, Irvinne, Calif. U.S.A.) was added. In some of the other perfusion studies, the solutions contained 1 mM thiourea (British Drug Houses) and [^{14}C]thiourea (2.5 μC/l from Radiochemical Centre, Amersham, Bucks).

Analytical methods

The initial solutions, effluent and plasma, were analysed for Na^+ and K^+ by flame photometry and Cl^- by coulometric titration using equipment supplied by Evans Electroselenum, Halstead, Essex. Polyethyleneglycol was analysed by the turbidometric method of Hyden[3]. Glucose was measured by a specific glucose oxidase method[4]. For radioactive counting, 1 ml of intestinal fluid or plasma was placed in 15 ml of scintillation fluid (750 ml toluene, 250 ml Triton, containing 4 g PPO per l) and counted in a Tracerlab liquid scintillation counter. The ^{22}Na counts were uncorrected for quench or counting efficiency, whereas the ^{14}C counts were obtained as dpm from computed quench curves. In some studies total bile salt concentration was measured in initial fluid and effluent by a steroid dehydrogenase method[5].

Calculations

In the closed loops, absorption of fluid was calculated as the difference between input volume and final loop volume, as measured by weighing. Bidirectional fluxes of

$^{22}Na^+$ were calculated from the formulae of Berger and Steele[6], which make allowances for the rapid exponential fall of specific activity during the experimental period and for any re-entry of absorbed $^{22}Na^+$ into the lumen.

In the perfusion studies, absorption rates of fluid and solute were calculated from standard formulae[7], and in each study a mean value was obtained from the three 20-min collection periods. The disappearance rate of [^{14}C]thiourea, calculated as for any other solute, can be used as a measure of net thiourea absorption because, being a foreign molecule, its specific activity will remain unchanged during absorption. Absorption will be underestimated if labelled solute diffuses back into the lumen. However, the magnitude of this is likely to be small, because final plasma ^{14}C activities were never more than 10% of the effluent activity, even after 2 h of perfusion. Similarly, the disappearance of [^{14}C]chenodeoxycholate has been used as an estimate of bile salt absorption. This also assumes that there is no change of luminal specific activity and therefore no exchange of bile salt across the mucosa. In a limited series of perfusions with 5 mM chenodeoxycholate, bile salt concentrations were measured chemically and isotopically and there was negligible change of specific activity: mean values for jejunum: in 1149, out 1167 dpm/μmole ($n = 6$) and for ileum: in 1159, out 1267 dpm/μmole ($n = 6$). In view of this, disappearance of isotope has been used as a measure of net absorption of bile salt.

Parametrical statistical methods have been used throughout. The significance of differences between mean values was assessed by the Student t test and correlation coefficients (r) and regression data for pairs of variables have been calculated from standard formulae[8].

RESULTS

Bidirectional movements of Na^+—closed loop studies

The net and bidirectional movements of Na^+ in jejunal and ileal closed loops are shown in Table I. The net results demonstrate that Na^+ absorption was completely inhibited in the jejunum by 2.5 mM deoxycholate and that marked Na^+ secretion occurred in the presence of 5 mM deoxycholate. The bidirectional flux studies showed no significant difference in the rates of Na^+ transport from lumen to plasma, whereas the movement in the opposite direction was significantly increased when the 5 mM deoxycholate result was compared with control ($P < 0.05$).

TABLE I

BIDIRECTIONAL Na^+ MOVEMENTS—CLOSED LOOP STUDIES

The units are in μequiv/g wet wt per 20 min (jejunum) or 30 min (ileum). Mean values $\pm$ 1 S.E. ($n = 6$) rats throughout. Negative sign indicates net transport from plasma to lumen. L→P = lumen to plasma. P→L = plasma to lumen.

Test solution	*Jejunum*			*Ileum*		
	Control	*2.5 mM deoxycholate*	*5 mM deoxycholate*	*Control*	*2.5 mM deoxycholate*	*5 mM deoxycholate*
Net transport	42.2 ± 3.8	−20.8 ± 16.5	−81.2 ± 7.4	120 ± 18.1	81.2 ± 4.1	37.3 ± 10.8
Flux L→P	273 ± 18.8	251 ± 13.9	246 ± 26.5	486 ± 58.0	441 ± 10.0	437 ± 53.0
Flux P→L	231 ± 18.3	272 ± 28.4	325 ± 33.4	351 ± 39.0	360 ± 26.0	396 ± 48.0

By contrast, in the ileum, 2.5 and 5 mM deoxycholate produced significant inhibition of net Na^+ absorption ($P < 0.01$) but did not abolish it completely. The flux studies did not demonstrate any significant effect on transport in either direction, although there was a tendency for lumen to plasma flux to decrease and plasma to lumen flux to increase with increasing concentrations of deoxycholate.

Perfusion studies in the jejunum

Effects of bile salts on mucosal transport and PD. The results are shown in Table II. 1 mM deoxycholate produced a significant inhibition of absorption of water ($P < 0.001$), glucose ($P < 0.01$), Na^+ ($P < 0.001$) and Cl^- ($P < 0.001$), and converted K^+ absorption to secretion (differences from zero transport, $P < 0.05$, $P < 0.01$, respectively). 5 mM deoxycholate totally abolished absorption of water Na^+ and Cl^- (differences from zero transport: $P > 0.3$, > 0.3, > 0.2, respectively) and further increased K^+ secretion (difference from 1 mM deoxycholate $P < 0.05$). Glucose absorption was further inhibited, but not abolished (difference from zero transport, $P < 0.001$). The effects of 5 mM chenodeoxycholate were somewhat different in that glucose absorption was rather less inhibited (difference from 5 mM deoxycholate $P < 0.01$; difference from control $P < 0.01$), and significant secretion of water occurred (difference from zero transport, $P < 0.05$).

The mucosal potential difference was significantly reduced by both 5 mM deoxycholate ($P < 0.001$) and 5 mM chenodeoxycholate ($P < 0.001$) to the same extent. However, in spite of abolition of Na^+ and water movement, the mucosa remained significantly polarised with serosal positivity (difference from zero potential, $P < 0.001$).

Effects of withdrawal of glucose from perfusion fluid. In view of the known stimulant effect of glucose on net Na^+ and water transport and on mucosal potential difference in rat jejunum *in vivo*[9] further perfusions were performed with a glucose-free solution. The results are shown in Table III. Absorption rates from the control solution of water, Na^+ and Cl^- and the PD, were significantly less than those shown in Table II ($P < 0.001$ in each case) confirming the stimulant effect of glucose on transport. In the glucose-free studies, 1 mM deoxycholate had no effect on the transport rates of water, Na^+, Cl^-, or K^+, whereas 5 mM deoxycholate abolished absorption of water, Na^+ and Cl^- and produced significant K^+ secretion (difference from zero transport, $P < 0.001$). Compared with the glucose containing solutions, 5 mM deoxycholate produced more profound impairment of water transport because significant secretion occurred (difference from zero transport, $P < 0.05$). In spite of the marked effect of 5 mM deoxycholate on Na^+ transport, the potential difference was unaltered.

These effects of both glucose and bile salts are illustrated graphically in relation to water transport in Fig. 1.

Correlations between individual ion and water transport rates. There was a highly significant positive correlation between transport rates of water and Na^+ ($r = 0.931$, $P < 0.001$) and water and Cl^- ($r = 0.905$, $P < 0.001$) and the relationship was unaffected by the presence or absence of glucose or bile salt. There was also a significant correlation between water and K^+ transport ($r = 0.86$, $P < 0.001$) (Fig. 2). Net K^+ secretion occurred in spite of little or no water secretion and a negligible concentration difference between infused fluid (4.0 mequiv/l) and plasma (mean 4.3

TABLE II

JEJUNAL PERFUSION STUDIES WITH GLUCOSE (20 mM)

Absorption rates are given in units/min per g wet wt of gut. Infusion rate 0.2 ml/min. Mean values ± 1 S.E., with number of rats in parentheses. Negative sign indicates net entry into the lumen. PD = potential difference in mV. N.D. = not determined.

Solution perfused	*Water* (*ml*)	*Glucose* (*μmoles*)	Na^+ (*μequiv*)	Cl^- (*μequiv*)	K^+ (*μequiv*)	*PD (mV)* (*serosa positive*)
Control	0.0340 ± 0.0023 (11)	1.825 ± 0.124 (11)	5.109 ± 0.230 (6)	4.355 ± 0.324 (6)	0.047 ± 0.017 (8)	8.85 ± 0.58 (10)
1 mM deoxycholate	0.0190 ± 0.0015 (7)	1.190 ± 0.139 (7)	2.928 ± 0.363 (7)	1.494 ± 0.270 (7)	−0.044 ± 0.011 (7)	N.D.
5 mM deoxycholate	0.0027 ± 0.0025 (8)	0.514 ± 0.072 (8)	0.564 ± 0.440 (6)	0.033 ± 0.025 (6)	−0.078 ± 0.009 (8)	5.44 ± 0.208 (8)
5 mM chenodeoxycholate	−0.0131 ± 0.005 (6)	1.094 ± 0.190 (6)	N.D.	N.D.	−0.096 ± 0.014 (6)	4.47 ± 0.241 (6)

TABLE III

JEJUNAL PERFUSION STUDIES WITHOUT GLUCOSE

Details as indicated in the legend to Table II.

Solution perfused	*Water* (*ml*)	Na^+ (*μequiv*)	Cl^- (*μequiv*)	K^+ (*μequiv*)	*PD (mV)* (*serosa positive*)
Control	0.0189 ± 0.0021 (10)	2.895 ± 0.219 (10)	2.297 ± 0.250 (10)	−0.004 ± 0.0048 (10)	4.42 ± 0.31 (6)
1 mM deoxycholate	0.0199 ± 0.0021 (6)	3.121 ± 0.310 (6)	2.388 ± 0.331 (6)	0.0195 ± 0.0072 (6)	N.D.
5 mM deoxycholate	−0.0027 ± 0.0009 (6)	−0.382 ± 0.195 (6)	−0.249 ± 0.131 (6)	−0.118 ± 0.0121 (6)	4.83 ± 0.284 (6)

TABLE IV

ILEAL PERFUSION STUDIES

Details as indicated in the legend to Table II.

Solution perfused	*Water* (*ml*)	Na^+ (*μequiv*)	Cl^- (*μequiv*)	K^+ (*μequiv*)	*PD (mV)* (*serosa positive*)
Control	0.0235 ± 0.0026 (7)	4.345 ± 0.375 (7)	4.296 ± 0.271 (7)	−0.0086 ± 0.0156 (7)	3.90 ± 0.53 (6)
5 mM deoxycholate	0.0154 ± 0.0036 (9)	2.802 ± 0.595 (7)	4.419 ± 0.305 (7)	−0.088 ± 0.0164 (9)	2.87 ± 0.273 (6)
5 mM chenodeoxycholate	0.0191 ± 0.0017 (6)	2.957 ± 0.298 (6)	3.770 ± 0.460 (6)	−0.099 ± 0.0272 (6)	3.27 ± 0.191 (6)

mequiv/l, range 3.9–4.7, $n = 8$). Effluent K^+ concentrations were always higher than those infused and usually higher than in plasma. Mean ratios of effluent to plasma K^+ concentrations were as follows: Control, 1.03; 5 mM deoxycholate, 1.08; 5 mM chenodeoxycholate, 1.04. These ratios are interpretable in terms of the PD across the mucosa (see Discussion).

Perfusion studies in the ileum

Effects of bile salts on mucosal transport and PD. The results are shown in Table IV. 5 mM deoxycholate and 5 mM chenodeoxycholate produced similar and partial inhibition of Na^+ transport ($P < 0.05$ and $P < 0.02$, respectively) but the changes in water absorption were not statistically significant ($0.2 > P > 0.1$). Significant Na^+ absorption occurred, compared with complete inhibition in the jejunum. Cl^- absorp-

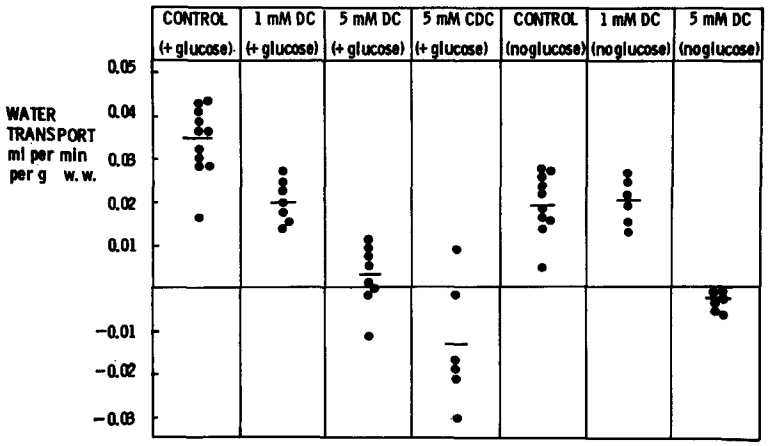

Fig. 1. Water transport in jejunal perfusions. Individual water absorption rates in ml/min per g wet wt during perfusion of rat jejunum with seven different solutions. DC = deoxycholate; CDC = chenodeoxycholate; w.w. = wet weight of gut; mean values shown. The standard errors are given in Tables II and III. Negative sign indicates net entry into lumen (secretion).

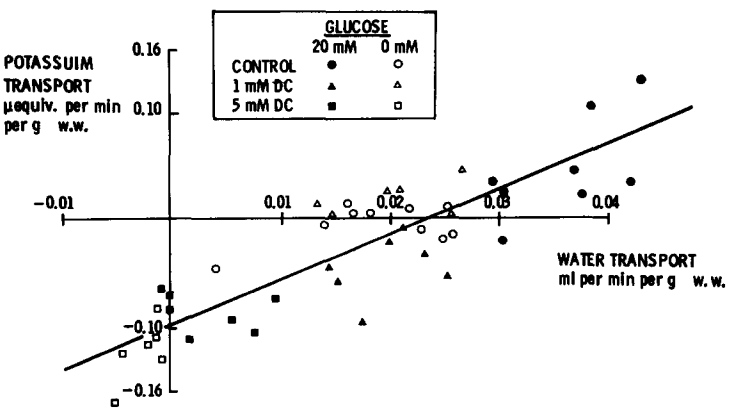

Fig. 2. Relationship between K^+ and water transport in jejunal perfusions. Correlation between individual transport rates of K^+ and water. Symbols indicate the different solutions perfused as described in the box. Line is the calculated regression line. DC = deoxycholate; w.w. = wet weight of gut. Regression equation: $y = 4.04x - 0.094$ ($r = 0.86$).

tion was unaffected by the presence of either bile salt and the mucosal potential differences were unchanged. Negligible K^+ transport in the controls (difference from zero transport, $P > 0.5$) was converted to significant secretion by 5 mM deoxycholate and chenodeoxycholate (differences from zero transport, $P < 0.001$, $P = 0.02$, respectively).

Correlation between individual ion and water transport rates. Again there was a significant positive correlation between water and Na^+ absorption ($r = 0.665$, $P < 0.01$) and water and Cl^- absorption ($r = 0.710$, $P < 0.01$) the relationship being unaffected by the presence or absence of bile salt. However, K^+ and water transport correlated poorly ($r = 0.225$, $P = 0.3$). As in the jejunum, luminal K^+ concentrations rose to exceed those in the plasma and mean values for effluent to plasma concentration ratios were: control, 1.08; 5 mM deoxycholate, 1.14; 5 mM chenodeoxycholate, 1.31.

Permeability studies in jejunum and ileum

Thiourea absorption rates in the perfusion studies are given in Table V. In the jejunum the disappearance of thiourea from solutions containing 5 mM deoxycholate was significantly reduced compared with controls in the presence ($P < 0.01$) or absence ($P < 0.01$) of glucose. Thiourea transport from control solutions was greater in the glucose containing then in the glucose-free perfusions, but this difference was not statistically significant ($0.1 > P > 0.05$). In the ileum, no change in the thiourea absorption was observed ($P > 0.3$).

In all the studies there was a significant correlation between individual transport rates of water and thiourea, which was unaffected by the presence or absence of glucose or bile salts. This is illustrated for the jejunal studies in Fig. 3. In the ileal studies a similar correlation was statistically significant ($r = 0.585$, $P < 0.05$).

The component of thiourea absorption due to diffusion (as opposed to solvent drag) can be assessed from the intercepts on the y axis in Fig. 3, *i.e.* thiourea transport at zero water flow. In the jejunal studies, the intercept on the y axis for the control solutions alone (with and without glucose) was 163.6 dpm/min ($r = 0.684$). This is not significantly different from the transport rates of thiourea from 5 mM deoxycholate solutions (Table V) at essentially zero flow ($P > 0.2$). In the ileal studies the regression equations for control and 5 mM deoxycholate solutions have been calculated separately and the intercepts on the y axis are virtually identical, being 235 ($r = 0.928$) and 228 ($r = 0.860$), respectively.

TABLE V

DISAPPEARANCE OF [^{14}C]THIOUREA

Jejunal and ileal perfusion studies as indicated. Units are in dpm/min per g wet wt. Mean value ± 1 S.E., number of rats in parentheses. Otherwise details as in the legend to Table II.

Solution perfused	*Jejunum*		*Ileum*
	With glucose	*No glucose*	*No glucose*
Control	328.6 ± 26.5 (11)	264.3 ± 21.2 (10)	323.5 ± 10.6 (7)
1 mM deoxycholate	269.1 ± 14.3 (8)	N.D.	N.D.
5 mM deoxycholate	199.8 ± 29.5 (8)	167.1 ± 22.8 (6)	355.8 ± 26.0 (9)

Absorption of [^{14}C]chenodeoxycholate

Absorption from 5 mM chenodeoxycholate solution was measured in jejunum and ileum using both closed loop and perfusion techniques (Table VI). Bile salt disappeared from the lumen to a much great extent in the closed loop than in the perfusion studies, and ileal absorption was more complete than jejunal in both types of study. These observations will be discussed in relation to some differences between results obtained by the two methods and in the two regions of the small intestine.

Histological changes

The histological observations in the closed loop studies have already been reported and illustrated[1]. Essentially the same appearances were seen in the perfusion

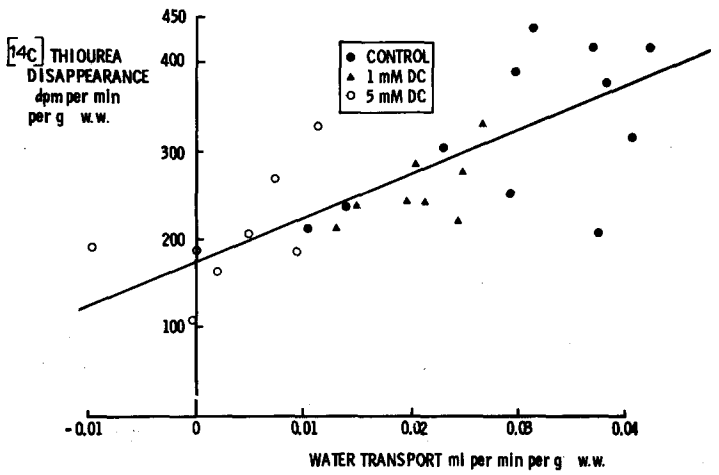

Fig. 3. Relationship between thiourea and water transport jejunal perfusions. Correlation between individual transport rates of thiourea and water. For clarity, only the values obtained with the glucose-containing solutions are shown. Other details as in the legend to Fig. 2. The regression equation for all observations (*i.e.* for glucose-free and glucose-containing solutions): $y = 4.6x + 177$ ($r = 0.746$).

TABLE VI

DISAPPEARANCE OF [^{14}C]CHENODEOXYCHOLATE FROM THE LUMEN—CLOSED LOOPS AND PERFUSION STUDIES

Percentage disappearance in the closed loops relates to the total absorptive period, *i.e.* 20 min (jejunum) or 30 min (ileum). In the perfusions, percentage disappearance relates to the amount infused in unit time. Percentage fall compares input and final luminal ^{14}C activity (closed loops) and infused and effluent ^{14}C activity (perfusions). Mean values shown with range in parentheses, $n = 6$ rats throughout.

Type of study	*Percentage disappearance of [^{14}C]chenodeoxycholate*		*Percentage fall in luminal ^{14}C activity*	
	Jejunum	*Ileum*	*Jejunum*	*Ileum*
Closed loops	75.5 (64.8–82.5)	90.3 (84.3–94.1)	78.2 (71.2–82.8)	88.1 (82.3–93.3)
Perfusions	29.5 (17.0–49.5)	56.3 (44.3–66.1)	38.1 (30.2–56.7)	48.6 (37.4–58.3)

experiments. No obvious abnormality was noted on light microscopy in the control perfusions and in those containing 1 mM deoxycholate. Definite damage to most of the villous tips was seen in loops perfused with either 5 mM deoxycholate or 5 mM chenodeoxycholate. Cells at the tips were swollen and misshapen or lost completely, whereas cells on the sides of the villi and in the crypts appeared quite normal and villous architecture was preserved. Similar changes were seen in both jejunum and the ileum.

DISCUSSION

Comparison of results obtained with the two methods

Using a perfusion technique our previous results obtained with closed loops[1] have been confirmed in that both deoxycholate and chenodeoxycholate inhibit the transport of glucose, water and electrolytes by the rat small intestine *in vivo*. As before, the ileum is relatively insensitive, although significant inhibition of electrolyte absorption was produced by 5 mM concentrations of both bile salts. In the jejunum, 1 mM deoxycholate inhibited absorption, whereas 5 mM concentrations abolished absorption (except of glucose) or produced slight secretion of fluid.

Some differences between the results obtained in the two studies deserve comment. Marked jejunal secretion of fluid seen in the closed loops in response to bile salts was not reproduced in the jejunal perfusions in the present study. This cannot be explained entirely by the tendency to overestimation of secretion, which is a feature of the closed loop system, because the magnitude of secretion is too great. Secretion might be underestimated in the perfusion studies by the apparent recovery of more than 100% of polyethyleneglycol in some of the studies. However, in the studies with 5 mM deoxycholate the mean steady state polyethyleneglycol recovery was 97.2%. We have no satisfactory explanation for these discrepant findings.

In recent jejunal perfusions in the hamster, Teem and Phillips[10] have reported the induction of fluid secretion by both conjugated and unconjugated dihydroxy bile salts: free deoxycholate producing secretion at concentrations of 2.5 mM and above, glycodeoxycholate producing secretion at 4 mM and above. They used an essentially similar perfusion system and the same non-absorbable marker. There are no other reported *in vivo* studies of small gut function available for comparison, but it is evident that both species and methological differences must be considered in the comparison of results obtained from different centres.

Another difference between our two studies was the effect of 1 mM deoxycholate on jejunal glucose absorption. In the present study glucose absorption was inhibited, confirming the observations of Gracey *et al.*[11] in similar studies on the rat jejunum. In the closed loops this was not observed and even at 5 mM concentrations of bile salt the inhibition of glucose absorption was relatively slight. This difference may be related to the very rapid disappearance of the bile salt from the closed loops compared with the perfusions (Table VI). Perfusion maintains a higher luminal bile salt concentration and this may be more important in determining inhibitory effects than the amounts of bile salt transported across the mucosa.

In the present study, 20 mM glucose had its expected effect of promoting jejunal absorption of water and electrolytes. This was not the case in the closed loops (unpublished observations), perhaps again because glucose disappeared rapidly

from the closed loops whereas perfusion maintained a higher luminal glucose concentration (mean falls in glucose concentration in control studies: closed loops, 22.8–1.2 mM ($n = 13$); perfusions 19.5–7.6 ($n = 11$)).

Effects of unconjugated dihydroxy bile salts on fluid and electrolyte transport

Deoxycholate and chenodeoxycholate produced essentially similar inhibitory effects, although some quantitative differences were observed. Chenodeoxycholate produced less inhibition of glucose absorption and greater effects on fluid transport (secretion rather than zero transport) than deoxycholate, but these differences were small and of dubious biological (as opposed to statistical) significance.

The quantitative relationships between individual transport rates of water and ions were unaffected by the presence of bile salts, so that the composition of fluid moving across the mucosa was similar in all studies. K^+ secretion in the jejunum can probably be attributed to a combination of solvent drag (Fig. 2) and the effects of the small transmucosal PD (Tables II and III). Final effluent to plasma K^+ concentration ratios were always greater than unity, but were less than those calculated for equilibrium on the basis of the Nernst equation[12]. Furthermore, the slope of the regression line of Fig. 2 was 4.04, which is very close to the mean luminal K^+ concentration. Since Na^+ transport is probably the major determinant of fluid and ion movement in the jejunum[13], it seems likely that bile salts directly affect this process. The Na^+ flux studies suggest that Na^+ movement from plasma to lumen is stimulated without inhibition of movement in the opposite direction. Similar observations were made in human colon perfusion studies[14], but not commented upon further. This will be discussed later in relation to the general problem of intestinal secretion of ions.

In the ileal studies, the magnitude of inhibition of water and Na^+ transport was strikingly less than in the jejunum, and the increased K^+ secretion cannot be explained in terms of solvent drag, because there was no correlation between water and K^+ movement. Effluent to plasma K^+ concentration ratios were higher than those observed in the jejunum and sometimes higher than equilibrium ratios to be expected from the Nernst equation (*e.g.* 5 mM deoxycholate; ratio 1.139, anticipated PD, 3.47; 5 mM chenodeoxycholate: ratio 1.303, anticipated PD, 7.07, *cf.* observed PD values in Table IV). Although bile salts may stimulate an active K^+ secreting mechanism, these differences are small and further studies with differing luminal K^+ concentrations would be required to clarify this point. Unlike the jejunum, Cl^- transport was unaffected by bile salts in the ileum. It is known that Cl^- can be transported independently of Na^+ in the ileum, perhaps by Cl^-–HCO_3^- exchange[15]. The relative stability of Cl^- transport in the rat ileum has previously been noted in relation to the effects of reduced luminal pH (ref. 16).

No previous reports, except our own, have commented on the marked differences between ileal and jejunal responses to bile salts. The bile salts were themselves absorbed more rapidly by the ileum as expected (Table VI), again suggesting that "toxicity" is not related to the amount of bile salt crossing the mucosal cells. The fall in bile salt concentration along the lumen was greater in the ileum, but the differnces were small and it seems unlikely that this could be responsible for the marked differences observed in fluid transport. If the ileal mucosa is intrinsically insensitive to luminal bile salts, this is in great contrast to the colon, although these two regions have many fundamental transport phenomena in common. In both experimental

animal and man marked inhibition of fluid transport or secretion can be produced in the colon by dihydroxy bile salts in the lumen at concentrations of 5 mM or more[14,17].

Effects of unconjugated dihydroxy bile salts on glucose and glucose-linked Na^+ transport in the jejunum

Although glucose and Na^+ transport are normally closely coupled[18], there is clearly a glucose-independent Na^+ transport system in the rat jejunum (Table III). Both these components of Na^+ transport are associated with an electrical gradient across the mucosa, the difference in PD between the glucose-containing and glucose-free perfusions (Tables II and III) presumably reflecting the potential generated by the glucose-dependent component. This component of PD was abolished by concentrations of bile salt which inhibit glucose transport, but bile salts had no effect on the glucose-independent PD even when Na^+ transport was completely inhibited. Furthermore, glucose-independent Na^+ transport was relatively insensitive to bile salt because it was unaffected by 1 mM deoxycholate, whereas glucose transport and presumably its related Na^+ transport were inhibited by this concentration (Tables II and III). This suggests that these bile salts may have two distinct effects on jejunal Na^+ transport—direct inhibition of glucose-dependent transport and an effect on glucose-independent transport which does not abolish the mucosal PD. This, coupled with the evidence suggesting increased Na^+ flux into the lumen, is compatible with the promotion of non-electrogenic movement of Na^+ (*plus* Cl^-) into the lumen without any interference with the normal electrogenic transport of Na^+ out of the lumen.

Mechanisms of the effects of bile salts on Na^+ and other transport systems

The present evidence cannot define how the bile salts produce their effects but some tentative conclusions are possible. It is well known that *in vitro* these bile salts, especially deoxycholate, produce severe histological damage associated with irreversible functional impairment[19,20]. This is not always the case *in vivo*, although damage to villous tips was produced by 5 mM concentrations of both bile salts, and similar findings have been reported in the hamster and guinea pig. Other studies in the rat have demonstrated little or no histological damage by 2 mM deoxycholate[21]. The functional effects *in vivo* do not appear to be closely related to histological damage. Thus in the present studies, similar damage was seen in jejunum and ileum and yet the functional effects in the two regions were quite different. No frank histological damage was seen with 1 mM deoxycholate and yet inhibition of all transport processes occurred. The effects on transport *in vivo* are reversible, as shown in our previous study[1], and also in the hamster jejunum[10] and in both the human and the canine colon[14,17]. Finally, the dissociation of effects on glucose and Na^+ transport seem incompatible with the concept of non-specific damage to the mucosa.

The studies with [^{14}C]thiourea suggest that mucosal permeability to small uncharged water-soluble molecules is not significantly affected by the presence of luminal bile salts, when solvent drag by bulk movement of water is taken into account. The mucosal cell membranes are considered to provide the barrier to the free movement of such molecules, but their exact route of transport (through 'pores' or between cells) is not known. The present findings suggest that this barrier is not affected by bile salts, again supporting the view that there is no gross damage to the

majority of mucosal cells. It is unlikely that changes in mucosal permeability *per se* would underly these altered fluid movements without some change in the driving force across the mucosa, whether osmotic, electrical or hydrostatic.

Surface effects of bile salts on the properties of lipid membranes would perhaps be anticipated by analogy with their 'detergent' effects on luminal lipids. It seems unlikely that such a surface effect would account for our observations, because the taurine conjugate of deoxycholate (an effective 'detergent') had little effect on mucosal transport of fluid in our previous study[1]. However, in the hamster small intestine and in the colon of several species, mucosal fluid transport can be inhibited by both conjugated and free dihydroxy bile salts, but not by the trihydroxy salts in concentration up to 10 mM[10,14,17]. It has been suggested that this difference is related to the fact that the trihydroxy bile salts are less surface active than the dihydroxy salts[14]. Our discrepant observations make it difficult to interpret the functional effects on transport in terms of the detergent properties of individual bile salts.

As already discussed, these bile salts appear to promote the secretion of water and ions into the jejunal lumen. Recent studies in experimental and human cholera have focused attention on the phenomenon of intestinal secretion and indirect evidence has accumulated to suggest that there may be a cyclic AMP mediated secretory mechanism in the intestinal mucosa[22]. The possibility that bile salts may stimulate this mechanism in some way is currently being investigated in this laboratory.

ACKNOWLEDGEMENTS

The authors wish to thank Messrs L. Ellam and A. Kingston for technical assistance, Mr R. Woodrough for help with the electrical apparatus and Dr A. M. Dawson for criticism and advice. Both authors gratefully acknowledge the financial support provided by the Wellcome Trust.

REFERENCES

1 J. T. Harries and G. E. Sladen, *Gut*, 13 (1972) in the press.
2 S. Feldman, M. Salvino and M. Gibaldi, *J. Pharmacol. Sci.*, 59 (1970) 705.
3 S. Hyden, *Ann. Swed. Agr. Coll.*, 22 (1955) 139.
4 A. StG. Hugget and D. A. Nixon, *Biochem. J.*, 66 (1957) 12P.
5 L. A. Turnberg and A. Anthony-Mote, *Clin. Chim. Acta*, 24 (1969) 253.
6 E. Y. Berger and J. M. Steele, *J. Gen. Physiol.*, 41 (1958) 1135.
7 G. E. Sladen and A. M. Dawson, *Clin. Sci.*, 36 (1969) 119.
8 *Documenta Geigy Scientific Tables*, J. R. Geigy, Basle, 1956, 5th edn, p. 31.
9 R. J. C. Barry, S. Dikstein, J. Matthews, D. H. Smyth and E. M. Wright, *J. Physiol.*, 171 (1964) 316.
10 M. V. Teem and S. F. Phillips, *Gastroenterology*, 62 (1972) 261.
11 M. Gracey, V. Burke and A. Oshin, *Scand. J. Gastroenterol.*, 6 (1971) 273.
12 J. S. Fordtran and J. M. Dietschy, *Gastroenterology*, 50 (1966) 263.
13 D. S. Parsons, *Brt. Med. Bull.*, 23 (1967) 252.
14 H. S. Mekhjian, S. F. Phillips and A. F. Hofmann, *J. Clin. Invest.*, 50 (1971) 1569.
15 K. A. Hubel, *Am. J. Physiol.*, 213 (1967) 1409.
16 B. Rousseau and G. E. Sladen, *Biochim. Biophys. Acta*, 233 (1971) 591.
17 H. S. Mekhjian and S. F. Phillips, *Gastroenterology*, 59 (1970) 120.
18 D. H. Smyth, *Phil. Trans. R. Soc. London, Ser. B*, 262 (1971) 121.
19 J. L. Pope, T. M. Parkinson and J. A. Olson, *Biochim. Biophys. Acta*, 130 (1966) 218.
20 T. S. Low-Beer, R. E. Schneider and W. O. Dobbins, *Gut*, 11 (1970) 486.
21 M. L. Clark, H. C. Lanz and J. R. Senior, *J. Clin. Invest.*, 48 (1969) 1587.
22 M. Field, *N. Engl. J. Med.*, 284 (1971) 1137.